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NOTES ON GEOGRAPHIC DISTRIBUTION

Discovery of a new host plant of *Pseudasphondylia tominagai* Elsayed & Tokuda, 2019 (Diptera, Cecidomyiidae)

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Abstract. Barely visible flower bud galls of *Deutzia floribunda* Nakai (Hydrangeaceae) were found in Kumamoto Prefecture, Kyushu, Japan. Morphological and molecular analyses indicated that the gall inducer is *Pseudasphondylia tominagai* Elsayed & Tokuda, 2019 (Diptera, Cecidomyiidae), previously known only to form galls on *Eleutherococcus spinosus* (L.f.) S.Y.Hu (Araliaceae) in Honshu, Japan. This study marks the first report of a species of *Pseudasphondylia* Monzen, 1955 on Hydrangeaceae, having host plants from different families and establishing *D. floribunda* as a new host record for *P. tominagai*.

Key words. Asphondyliini, Asphondyliina, *Deutzia*, gall midge, Hydrangeaceae, phylogeny

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INTRODUCTION

The gall midge genus *Pseudasphondylia* Monzen, 1955 (Cecidomyiidae, Cecidomyiinae, Asphondyliini) is known from 14 described species distributed across the eastern Palearctic and Oriental regions (Gagné and Jaschhof 2021; Matsuda et al. 2021; Lin et al. 2022; Elsayed et al. 2023). This wide distribution is accompanied by a broad range of host plants from at least 11 angiosperm families (Gagné and Jaschhof 2021; Matsuda et al. 2021; Lin et al. 2022). *Pseudasphondylia* species inducing leaf galls are univoltine, while partial populations in some species enter extended diapause and exhibit two-year life cycle (Yukawa 1974, 1987; Takasu and Yukawa 1984; Tokuda and Yukawa 2005; Yukawa and Tokuda 2021; Matsuda et al. 2021). Mature larvae of most known species overwinter in galls on host plants or on the ground, pupate in galls, and the adults emerge directly from galls in all known species, except *P. ishigakiensis* Elsayed, 2023 of which some pupae were reported to drop to the ground (Elsayed et al. 2023). Other *Pseudasphondylia* species, especially those associated with host flower buds, have multivoltine life cycles with potential host-alternation, but their alternative hosts have not yet been clarified (Tokuda and Yukawa 2005; Elsayed et al. 2023). Further faunistic and ecological surveys are necessary to confirm the life cycle of multivoltine species as well as to shed light on the evolution of life history strategies.

Recently, a species of *Pseudasphondylia* was found developing in the flower buds of *Deutzia floribunda* Nakai (Hydrangeaceae) (Figure 1A, B) in Kumamoto Prefecture, Kyushu, Japan (Figure 2). To identify this species, we examined the larval and pupal morphology, and we compared its DNA sequences to known *Pseudasphondylia* species in Japan.

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METHODS

Flower buds of *Deutzia floribunda* (Figure 1A, B) were collected near Ozu Town, Kumamoto Prefecture, Kyushu, Japan (Figure 2) in late June 2023. Some buds, with compact appearance and thickened petals, were cut open in the field to confirm the presence of gall midges (Figure 1C). Some galled buds were dissected under a stereoscopic microscope to obtain mature larvae and pupae, while the remaining buds were kept in plastic bags to obtain adults. A few pupae were dropped in plastic bags before maturation (Figure 1D) and were transferred to rearing cups designed by Elsayed et al. (2018a), but no adults emerged successfully due to fungal infection. The specimens obtained were preserved separately in 99.5% ethanol for morphological examinations and molecular analyses.

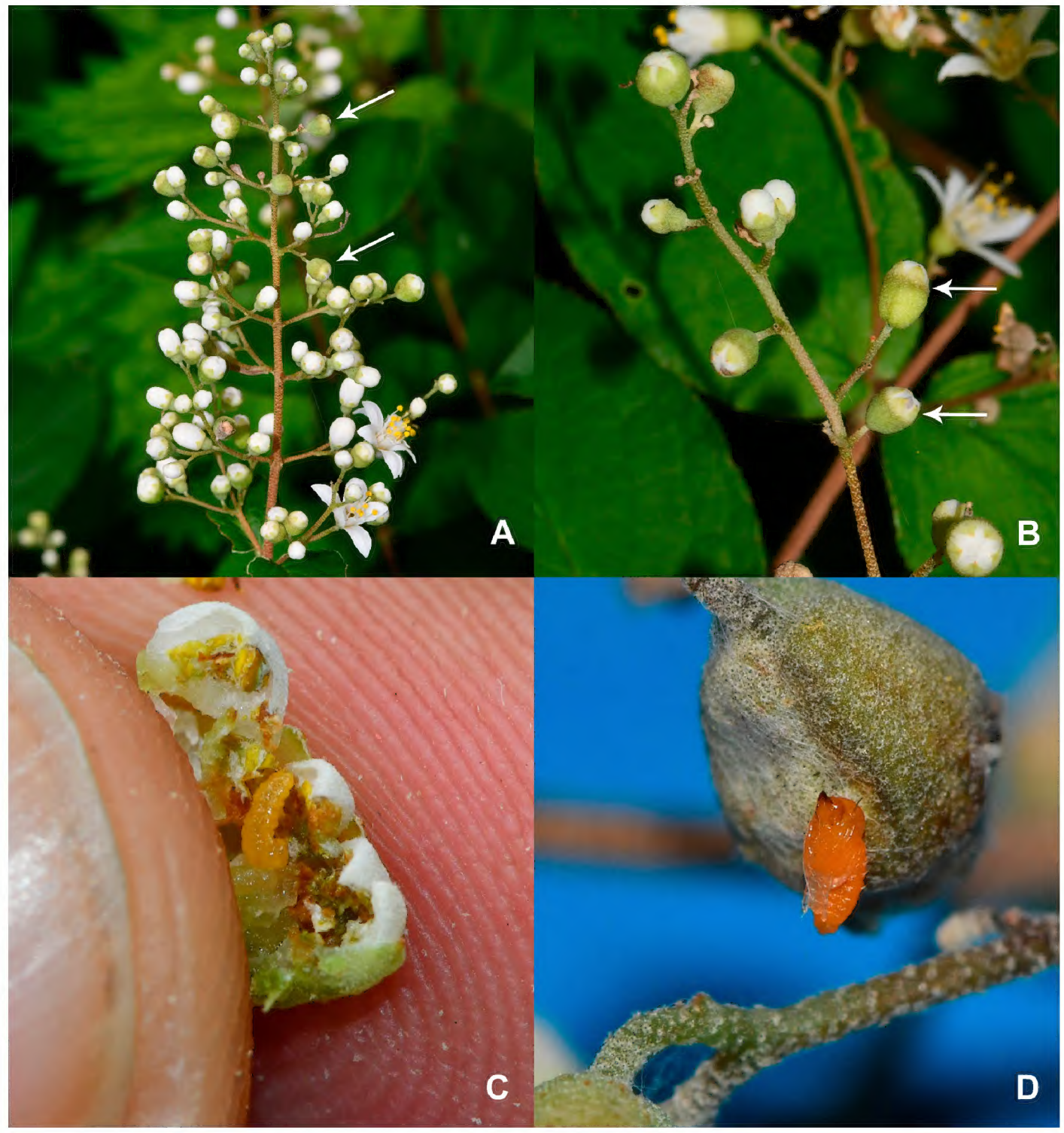

Figure 1. *Pseudasphondylia tominagai* Elsayed & Tokuda developing in flower buds of *Deutzia floribunda* (Hydrangeaceae). **A, B.** Slightly deformed galled flower buds of *D. floribunda* (arrows). **C.** Yellow larva and its associated fungus in a cut-open flower bud. **D.** Emerged young pupae of *P. tominagai*.

Some larval and pupal specimens were mounted on slides following the technique outlined in Gagné (1994), with the modifications by Elsayed (2023). The slide-mounted specimens were examined under a bright-field and phase-contrast microscope (CX43, Olympus, Tokyo). Generic identification was done based on the diagnosis of *Pseudasphondylia* given by Elsayed et al. (2023) and Tokuda and Yukawa (2005). Species identification was done based on Elsayed et al. (2019a). Materials examined are deposited in the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Japan (ELKU). The distribution map (Figure 2) was produced based on an image from Google Earth Pro v. 7.3.6.9796.

Total DNA was extracted from each of two pupae using DNeasy Blood and Tissue Kit (Qiagen, Tokyo, Japan) and the Folmer fragment (472 bp) of the mitochondrial COI gene was sequenced and aligned following Elsayed et al. (2017a). The primer set used in the analysis is J-1718 (5′–GGA GGA TTT GGA AAT TGA TTA GTT CC–3′) (Simon et al. 1994) and COIA (5′–CCC GGT AAA ATT AAA ATA TAA ACT TC–3′) (Funk et al. 1995). The sequences obtained were compared using MEGA 11 (Tamura 2021) and deposited in the DNA Data Bank of Japan (DDBJ), European Molecular Biology Laboratory (EMBL), and GenBank nucleotide sequence databases as accession numbers LC844092 and LC844093.

In addition to the sequence data obtained, sequences of 11 species of *Pseudasphondylia* species lodged in GenBank were used as ingroup taxa: *P. rokuharensis* Monzen, 1955 (LC538357), *P. kiritanii* Tokuda & Yukawa, 2005 (LC538356) (Lin et al. 2020), *P. matatabi* (Yuasa & Kumazawa, 1938) (AB085873) (Yukawa et al. 2003), *P. neolitseae* Yukawa, 1974 (AB334237) (Tokuda et al. 2008), *P. elaeocarpi* Tokuda & Yukawa, 2005 (LC621306), *P. tominagai* Elsayed & Tokuda, 2019 (LC621302), *P. saohimea* Matsuda, Elsayed & Tokuda, 2020 (LC621304) (Matsuda et al. 2021), *P. kiwiphila* Lin, Tokuda & Yang, 2022 (LC710538) (Lin et al. 2022), and *P. ishigakiensis* (LC761829) (Elsayed et al. 2023). GenBank sequences of five distantly related species were used as outgroup taxa: *Asphondylia tojoi* Elsayed & Tokuda, 2018 (LC426406) (Elsayed et al. 2018b), *A. sphaera* Monzen, 1937 (AB197955) (Uechi and Yukawa 2006), *Ampelomyia conicocoricis* Elsayed & Tokuda, 2019 (LC422078) (Elsayed et al. 2019b), *Gephyraulus zewaili* Elsayed & Tokuda, 2017 (LC270942) (Elsayed et al. 2017b) and *G. tokudai* Elsayed, 2022 (LC685415) (Elsayed and Kawakita 2022). These sequences were aligned using ClustalW algorithm in MEGA 11 (Tamura 2021). The phylogenetic relationships were inferred using the maximum-likelihood (ML) method in MEGA 11 based on 1000 bootstrap replications and the GTR+G+I model determined by modeltest in RAxML GU 2.0.6 (Edler et al. 2021).

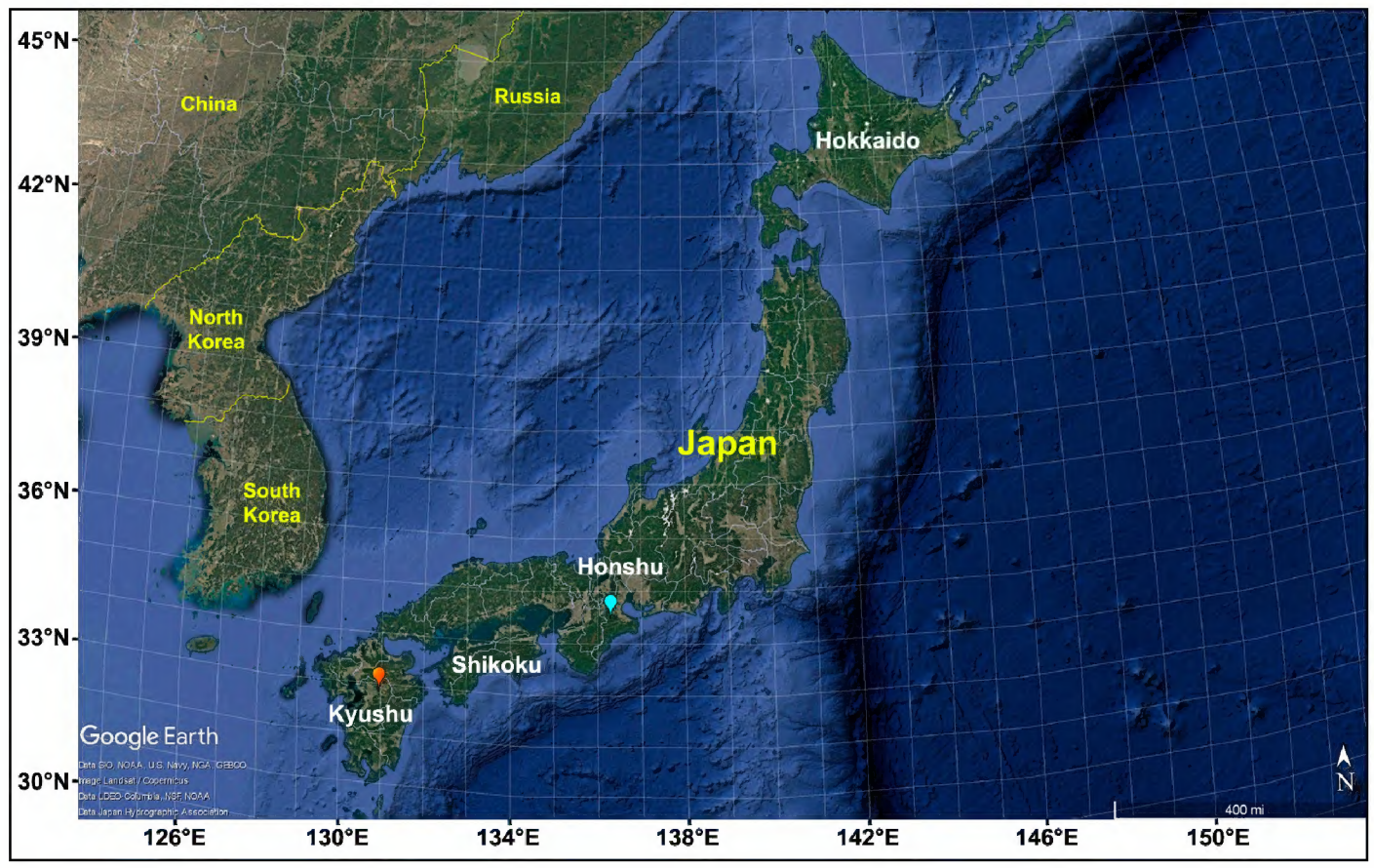

Figure 2. Map of the main islands of Japan showing the distribution points of *Pseudasphondylia tominagai* (edited Google Earth Pro image). The blue marker shows previously known point of occurrence in Mie Prefecture, Honshu (Elsayed et al. 2019a), while the red marker indicates the newly recorded distribution in Kumamoto Prefecture, Kyushu.

RESULTS

Pseudasphondylia tominagai **Elsayed & Tokuda, 2019a**
Figures 1A–D, 3

New records. JAPAN – Kyushu • Kumamoto Prefecture, Ozu Town; 32°54′59.7″N, 130°57′35.1″E; collected 24.VI.2023; A.K. Elsayed & M.K. Elsayed leg.; *Deutzia floribunda* (Hydrangeaceae) flower buds; dissected. 26.VI.2023; 6 third instar larvae, 6 pupae, ELKU, on slides, nos. Cecid. ET0123, ET0124.

Identification. The morphological examination of the mature larvae and pupae from the flower buds of *D. floribunda* indicated that the gall midge is closest to *P. tominagai* and *P. kiritanii* because of the following shared characters: quadridentate spatula of which the outermost teeth are shorter than the innermost, four setose papillae on the larval terminal segment, spiracles on pupal abdominal segments II–VI, each is about 0.5 times as long as the prothoracic spiracle, 4–5 rows of spines on the pupal abdominal terga II–VIII; and two setose and two asetose dorsal papillae on the pupal abdominal terga I–VII (Tokuda and Yukawa 2005; Elsayed et al. 2019a). The diagnostic characteristics to separate *P. tominagai* and *P. kiritanii* occur in male hypoproct and female ovipositor, but we could not confirm them due to the unavailability of adult specimens of the gall midge associated with flower buds of *D. floribunda*.

In the molecular phylogenetic analysis the sequences of the gall midge obtained from *D. floribunda* were 100% identical and formed a strongly supported clade with *P. tominagai* and *P. kiritanii* (Figure 3), corroborating their morphological similarity. The sequence divergence between the gall midge obtained from *D. floribunda* and *P. tominagai* was 0.6%, but 2.1% with *P. kiritanii*. Based on this high COI similarity, we conclude that the gall midge specimens collected from *D. floribunda* belong to *P. tominagai*.

Pseudasphondylia tominagai was previously known to induce galls on *Eleutherococcus spinosus* (Araliaceae) in Mie Prefecture, Honshu, Japan (Figure 2; Elsayed et al. 2019a). Its discovery on *D. floribunda* in Kyushu represents a new host and a new distribution record, and this strongly suggests that the gall midge alternates the host plant. Also, this is the first report of a *Pseudasphondylia* species associated with Hydrangeaceae and having host plants of different plant families.

DISCUSSION

Given the low genetic distance between *Pseudasphondylia tominagai* and *P. kiritanii* and their development into flower bud galls, one might consider *P. tominagai* a synonym of *P. kiritanii*. However, they exhibit slight morphological differences in the male terminalia and female ovipositor (Elsayed et al. 2019a). Further research is needed to investigate their genetic distances among various populations and to assess the significance of their slight morphological differences to determine whether these species should indeed be synonymized.

The larvae of *P. tominagai* develop in the flower buds of *Eleutherococcus spinosus* (L.f.) S.Y.Hu (Araliaceae) in April to late May. The adults emerge in late May after the flowering season of *E. spinosus* has ended and there are no available oviposition sites on the host (Elsayed et al. 2019a). According to our new host record, *P. tominagai* is possible to move to *D. floribunda* which flowers from June to August. In our

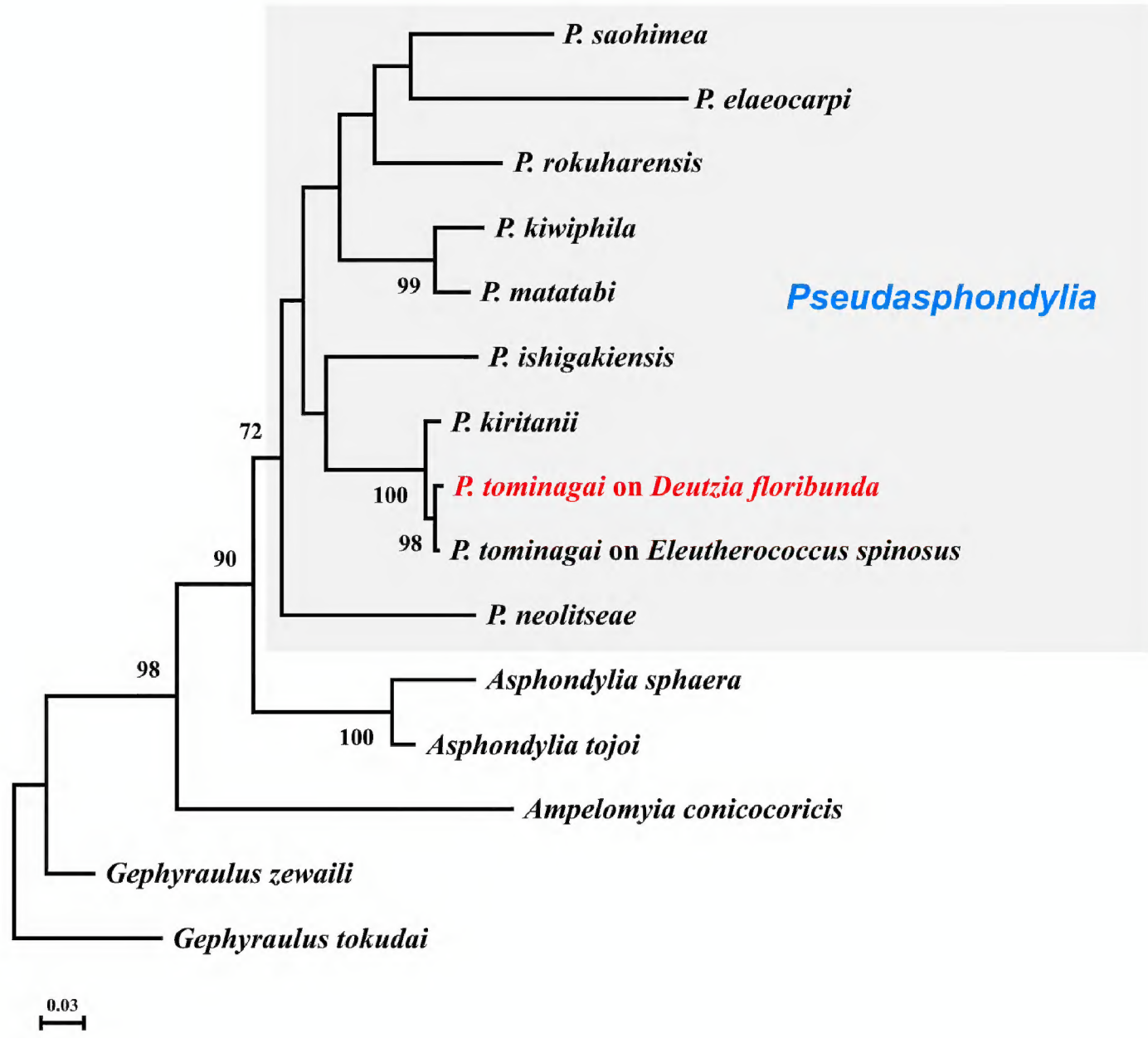

Figure 3. A maximum-likelihood phylogenetic tree based on partial COI sequences. Bootstrap values are indicated for nodes that gained >50% support.

observations, larvae were not present in the overwintering buds of both hosts and some young pupae of *P. tominagai* crawl out of the galls of *D. floribunda* (Figure 1D), possible to move to the ground under natural conditions. The pupal emergence from galls is rather unusual behavior in gall midges that has been reported recently in *P. ishigakiensis* (Elsayed et al. 2023) and is known in at least three species, namely *Contarinia negundinis* (Gillette, 1890), *Resseliella lavandulae* (Barnes, 1953), and *Hasegawaia sasacola* Monzen, 1937 (Wilson 1966; Sunose 1978; Coutin 1982; Gagné 1989). Based on ecological information of *P. tominagai*, two alternative life history scenarios can be suggested for *P. tominagai*: first, *P. tominagai* is polyphagous and may utilize additional host plant(s) after the flowering season of *D. floribunda*; or second, the emerged pupae overwinter in the ground until the next flowering season, although pupal overwintering is uncommon in gall midges and has not yet been reported in Asphondyliini (Tokuda 2012; Yukawa and Tokuda 2021). Although further investigations are needed to determine the complete life history of *P. tominagai*, the present results strongly suggest the host-alternating life history of this gall midge, which has been suspected in this genus but never demonstrated.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: AKE, MT. Data curation: AKE. Formal analysis: AKE. Funding acquisition: AKE. Investigation: AKE. Methodology: AKE, MT, SN. Visualization: AKE. Project administration: AKE. Software: AKE. Writing – original draft: AKE, MT, SN. Writing – review and editing: AKE, MT.

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Data availability

All data that support the findings of this study are available in the main text.

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